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14. ABSTRACT

Tumor biology is a recognized determinant of tumor behavior, including growth rate, motility and metastatic potential, and therapeutic resistance. This project was funded to investigate the regulation and expression of an excellent marker for aggressive breast tumors: RhoC-GTPase. When overactive, RhoC transforms mammary epithelial cells into a highly motile and invasive phenotype. We hypothesize that RhoC overexpression may be regulated by the transcription factor NF-kappa B and that at the same time RhoC is overexpressed the tumor also acquires therapy resistance. The objective of this study is to utilize existing breast cancer cohorts with tumor tissue and treatment response data available to assess the correlation between NF-kappa B and RhoC, individually and in combination, to treatment response.

The specific aims of the project are to determine 1) if RhoC and NF-kappa B are correlated; 2) if RhoC and NF-kappa B are associated, individually and in combination, with aggressive breast cancer; and 3) if NF-kappa B and RhoC are associated with therapy resistance.

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INTRODUCTION

This study was funded to assess the relationship of RhoC and NF-kappa B to aggressive, metastatic, and therapy-resistant breast cancer. Inflammation is currently being considered a key component of cancer initiation and progression. [1] Many proinflammatory stimuli act through an NF-kappa B dependent signaling pathway. It is likely that RhoC, which is recognized as an important marker of breast cancer, aggressiveness is also regulated by NF-kappa B.

The tumor microenvironment is exceptionally critical in our understanding of cancer development. Recent research suggests that chronic inflammation is triggered during tumor growth. The tumor associated inflammation contributes greatly to the tumor microenvironment and perhaps even to tumor macroenvironment. Tumor-associated macrophages (TAMs) secrete numerous signaling molecules that interact with tumor and stromal cells. Secretion of TNF-α by TAMs activates NF-kappa B within the tumor cell through the receptor tyrosine kinase pathway.

Rho-GTPases are members of the Ras-superfamily. Activation of Rho promotes both the bundling of actin filaments with myosin II filaments into stress fibers and the clustering of integrins and associated proteins to form focal contacts. [2] Experiments conducted within the Merajver laboratory have shown an active role for RhoC in rearrangement of the cytoskeleton in cell motility and invasion.

We hypothesize that NF-kappa B is a transcription factor for the RhoC gene and leads to overexpression of cellular RhoC protein. As more is learned about these tumor markers, the potential exists for improved clinical diagnosis, prognosis, and treatment. The purpose of the study is to understand the regulation of RhoC gene expression in an epidemiological setting. A clear understanding of the genetic and cellular mechanisms involved in modulating a highly metastatic phenotype is expected to aid in diagnosis and treatment.

The scope of this work includes the identification and collection of patient information from Henry Ford Health system (HFHS). HFHS is an integrated health system that offers a diverse, population-based patient population from which strong epidemiologic studies can be built. Patient information, including diagnostic and recurrence data, will be combined with gene expression data. Molecular and statistical analysis will occur in collaboration with the University of Michigan.

BODY

In the third 12 months of the project, we projected that we would prepare the main study manuscript. Below are the objectives from the original statement of work.

Task 4. Prepare manuscript

a. Complete final data analyses and tables
b. Prepare draft manuscripts
c. Mentor and collaborators review of draft manuscripts
d. Prepare final manuscripts and submit for publication

The complete dataset (abstracted medical record data with TMA data) is expect to be established within the next 6 months. At that time we will be able to begin the analysis of the data for key results. Although all the pathological samples have been collected and transferred to the University of Michigan, completion of the TMA's was delayed due to medical leave of our participating pathologist, Dr. Kleer. Since Dr. Kleer's return, she has spent many hours training Ms. Alford in the correct identification of tumor sections in existing pathological specimens. Therefore, progress on this effort is proceeding rapidly and we plan to have the TMA's completed by the end of summer. This would allow for a completed dataset, analysis and preparation of the primary manuscript by the end of our no-cost extension.

Other research activities, supported in part by funding from this grant, have included working with a multi-institutional collaboration between Henry Ford Health System, University of Michigan, Wayne State University, and Oakwood Hospitals. This collaboration is focused on cancer epidemiology research within the significant Arab American population in metro-Detroit. Arab women have been reported to have an increased incidence of inflammatory breast cancer (IBC), a particularly aggressive form of breast cancer. RhoC has been shown to be amplified in inflammatory breast cancer and may likely be the key to IBC's aggressive phenotype. [1]

Also supported in part by funding from this grant, Ms. Alford recently attended the American Association of Cancer Research's special conference on Approaches to Complex Pathways in Molecular Epidemiology. This conference provided excellent information on bioinformatic approaches to understanding molecular relationships like the one we are investigating in this proposal.

KEY RESEARCH ACCOMPLISHMENTS

- Acquiring all pathological specimens and transferring them to University of Michigan
- Transfer of samples to the University of Michigan
- Investigator's training to recognize tumor sections in pathological specimens

REPORTABLE OUTCOMES

Reportable outcomes are expected for the next review.

CONCLUSIONS

Exciting research has surfaced in the past year regarding the tumor microenvironment and breast cancer metastasis. Areas which our genes of interest are likely to play a significant role. Despite this, no one else is currently investigating our genes of interest and their role in an aggressive breast cancer phenotype. As clinical oncology looks more to targeted therapy for treatment options, our work will hopefully be able to provide valuable insight for drug development.

REFERENCES

[1] van Golen KL, Davies S, Wu ZF et al. A novel putative low-affinity insulin-like growth factor-binding protein, LIBC (lost in inflammatory breast cancer) and RhoC GTPase correlate with the inflammatory breast cancer phenotype. Clin Cancer Res 1999;5:2511-9.